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03/27/03 J1132 U.S. PTO

03-28-03 60458162 .032703

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J1050 U.S. PTO 60/458162 03/27/03

Docket No. 3882-P03143US00	Type a plus sign (+) inside this box

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TITLE OF THE INVENTION (200 characters max)

METHODS FOR COMBINATORIAL DRUG STRATEGY FOR THE TREATMENT OF CANCER

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METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

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Applicant claims Small Entity Status	

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

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☐ Yes, the name of the U.S. Government agency contract number is: ____________

Respectfully submitted
DANN, DORFMAN, HERRELL AND SKILLMAN
A Professional Corporation

By Kathleen D. Rigaut
Kathleen D. Rigaut, Ph.D., J.D.
PTO Reg. No. 43,047

☐ Additional inventors are being named on separately numbered sheets attached hereto

METHODS FOR COMBINATORIAL DRUG STRATEGY FOR THE TREATMENT OF CANCER

By George C. Prendergast
Alexander J. Muller
James DuHadaway

FIELD OF THE INVENTION

This invention relates to the fields of oncology and chemotherapy. Specifically, the invention provides novel materials and methods for a combinatorial drug strategy for the treatment of cancer.

BACKGROUND OF THE INVENTION

Several publications and patent documents are cited in this application in order to more fully describe the state of the art to which this invention pertains. The disclosure of each of these citations is incorporated by reference herein.

Tumors characteristically express atypical, potentially immunoreactive antigens that are collectively referred to as tumor antigens. Accumulating evidence suggests that the failure of the immune system to mount an effective response against progressively growing tumors is not attributable to a lack of recognizable tumor antigens. Immunosuppression by tumors is poorly understood and mechanisms by which tumors may escape immune surveillance are poorly explored. Recently, it has been shown that cytotoxic T cells become tolerized by a reduction in local concentrations of tryptophan that are elicited by indoleamine 2,3-dioxygenase (IDO) activity.

IDO is an oxidoreductase that catalyzes the rate-limiting step in tryptophan catabolism. This enzyme is structurally distinct from tryptophan dioxygenase (TDO), which is responsible for dietary tryptophan

catabolism in the liver. IDO is an IFN-γ target gene that has been suggested to play a role in immunomodulation (Mellor and Munn (1999) Immunol. Today. **20**:469-473). Elevation of IDO activity depletes the levels of tryptophan in local cellular environments. In antigen-presenting cells, where IDO is regulated by IFN-γ, activation of IDO blocks activation of T cells. Two characteristics of T cells lend to this block in activation by IDO activity: 1) T cells are especially sensitive to tryptophan depletion and 2) T cells must undergo 1-2 rounds of cell division to become activated. In this way, IDO has been proposed to inhibit T_H1 responses that promote cytotoxic T cell development.

The main evidence for the role of IDO in immunosuppression is demonstrated by the ability of 1-methyl-tryptophan (1MT), a specific and bioactive IDO inhibitor (Cady and Sono (1991) Arch. Biochem. Biophys. **291**:326-333), to elicit MHC-restricted and T cell-mediated rejection of allogeneic mouse concepti (Mellor et al. (2001) Nat. Immunol. **2**:64-68; Munn et al. (1998) Science. **281**: 1191-1193). This effect is consistent with the high levels of IDO expression in placental trophoblast cells (Sedlmayr et al. (2002) Mol. Hum. Reprod. **8**:385-391).

Significantly, IDO activity has been shown to be elevated frequently in human tumors and/or in cancer patients (Yasui et al. (1986) Proc. Natl. Acad. Sci. USA. **83**:6622-6626; Taylor and Feng (1991) FASEB J. **5**:2516-22). Since IDO can modulate immune responses, one logical implication is that IDO elevation in cancer may promote tumor immunosuppression (Mellor and Munn (1999) Immunol. Today. **20**:469-473; Munn et al. (1999) J. Exp. Med. **189**:1363-1372; Munn et al. (1998) Science. **281**:1191-1193). This possibility is supported by the

observation that many cancers, including breast cancer, are characterized by a loss of beneficial immune functions that can limit malignant development. For example, T_H1 responses that promote the production of cytotoxic T cells (a process guided by IFN-γ) are suppressed during cancer progression. A resultant hypothesis of this data was that if IDO drives cancer progression by blunting T cell activation, then IDO inhibition in animals should blunt tumor growth by reversing IDO-mediated immunosuppression. However, delivery of the IDO inhibitor 1-methyl-tryptophan (1MT) only retarded and did not prevent tumor growth in a mouse model (Friberg et al. (2002) Int. J. Cancer **101**:151-155; US Patent 6,482,416).

SUMMARY OF THE INVENTION

In accordance with the present invention, a method of treating malignancy is provided. Specifically, methods for the combination drug treatment of an IDO inhibitor and a cytotoxic chemotherapeutic agent is provided. Pharmaceutical compositions consisting of an IDO inhibitor and a cytotoxic chemotherapeutic agent for the treatment of tumors are also provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph of the results from an *in vitro* biochemical assay for screening of IDO inhibitor candidates. Data is provided relative to the amount of kynurenine produced in the absence of inhibitor.

Figures 2A and 2B are graphs of the results from the cell-based assay for screening of IDO inhibitor candidates. In Figure 2A, data is provided relative to the amount of kynurenine produced in the absence of inhibitor. In Figure 2B, the data is presented in terms of fluorescence, which is indicative of kynurenine

production (i.e., IDO activity). Cells were either transfected with an empty expression vector (vector) or an expression vector containing the cDNA of IDO.

Figure 3 provides graphs of the thiohydantoin derivatives of indoleamine in the cell-based assay for screening of IDO inhibitor candidates. The cells were transfected with empty expression vectors (vector) or with expression vectors which contain IDO or TDO. For comparison, cells transfected with the IDO expression vector were also assayed in the presence of 1MT.

Figure 4 is a chart of certain IDO inhibitors, their structures, and their ability to inhibit IDO and TDO activity at a concentration of 250 μM in a cell-based assay.

Figure 5 provides graphs demonstrating the toxicity of certain IDO inhibitors of neoplastically transformed breast (top panel) or prostate (bottom panel) cancer cells. Cells were either untreated (Untx) or treated with 100 μM of inhibitor.

Figure 6 is a graph illustrating the fold change in tumor volume of MMTVneu mice either mock treated (untreated) or treated with 1MT, paclitaxel (Taxol®), 1MT and paclitaxel (Taxol®), cisplatin, or 1MT and cisplatin. Each data point was determined from an individual mouse and the bars indicate the mean of the data points as listed at the bottom of the graph.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, methods of employing inhibitors of IDO for the treatment of cancer are provided. These inhibitors are described in Example 1. All of the compounds identified as IDO inhibitors were obtained from Sigma (St. Louis, MO) with the exception of indole 3-carbinol, 3,3'-diindolylmethane, brassinin, epigallocatechin gallate

which were obtained from LKT laboratories (St. Paul, MN) and propenyl-TH-DL-tryptophan (propTH-trp) which was obtained from Asinex (Moscow, Russia). Many of these inhibitors display significantly improved potency over 1MT. Utilization of these IDO inhibitors in place of 1MT or other IDO inhibitors in methods and compositions already known in the art is contemplated in the instant invention.

Additionally, it has been discovered that the combination treatment of an IDO inhibitor with a cytotoxic chemotherapeutic agent provides an unexpectedly effective means of treating tumors.

Cytotoxic chemotherapeutic agents of the instant invention include, but are not limited to: placitaxel (Taxol®), cisplatin, docetaxol, carboplatin, vincristine, vinblastine, methotrexate, cyclophosphamide, CPT-11, 5-fluorouracil (5-FU), gemcitabine, estramustine, carmustine, adriamycin (doxorubicin), etoposide, arsenic trioxide, irinotecan, and epothilone derivatives. Taxol® and cisplatin were obtained from Hanna Pharmaceuticals (Wilmington, DE).

IDO inhibitors of the instant invention include, but are not limited to, previously established IDO inhibitors such as: 1-methyl-tryptophan, β-(3-benzofuranyl)-DL-alanine, β-(3-benzo(b)thienyl)-DL-alanine, and 6-nitro-L-tryptophan; and the novel IDO inhibitors described in the instant invention: indole 3-carbinol, 3,3'-diindolylmethane, brassinin, epigallocatechin gallate, 5-Br-4-Cl-indoxyl 1,3-diacetate, 9-vinylcarbazole, acemetacin, 5-bromo-DL-tryptophan, 5-bromoindoxyl diacetate, phenyl-TH-DL-trp, propenyl-TH-DL-trp, and methyl-TH-DL-trp.

As used herein, the phrase "effective amount" of a compound or pharmaceutical composition refers to an amount sufficient to modulate tumor growth or metastasis

in an animal, especially a human, including without limitation decreasing tumor growth or size or preventing formation of tumor growth in an animal lacking any tumor formation prior to administration, i.e., prophylactic administration.

Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers, for example to a diluent, adjuvant, excipient, auxilliary agent or vehicle with which an active agent of the present invention is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

A pharmaceutical composition of the present invention can be administered by any suitable route, for example, by injection, by oral, pulmonary, nasal or other forms of administration. In general, pharmaceutical compositions contemplated to be within the scope of the invention, comprise, inter alia, pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions can include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80),

anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc., or into liposomes. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of components of a pharmaceutical composition of the present invention. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which are herein incorporated by reference. A pharmaceutical composition of the present invention can be prepared, for example, in liquid form, or can be in dried powder, such as lyophilized form. Particular methods of administering such compositions are described infra.

In yet another embodiment, a pharmaceutical composition of the present invention can be delivered in a controlled release system, such as using an intravenous infusion, an implantable osmotic pump, a transdermal patch, liposomes, or other modes of administration. In a particular embodiment, a pump may be used [see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)]. In another embodiment, polymeric materials can be used [see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Press: Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley: New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)]. In yet another embodiment,

a controlled release system can be placed in proximity of the target tissues of the animal, thus requiring only a fraction of the systemic dose [see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)]. In particular, a controlled release device can be introduced into an animal in proximity of the site of inappropriate immune activation or a tumor. Other controlled release systems are discussed in the review by Langer [Science 249:1527-1533 (1990)].

The MMTVneu transgenic "oncomouse" model of breast cancer was used to measure the effects of IDO inhibition and cytotoxic chemotherapeutic agents on tumor pathophysiology. The MMTVneu transgenic mouse develops aggressive tumors of the mammary gland that resemble poorly differentiated human ductal carcinomas. In the MMTVneu mouse model, breast cancer is initiated by tissue-specific expression of a mutant form of the HER-2/Neu gene that is activated frequently in aggressive human breast ductal carcinomas. HER-2 is a member of the EGF-R family of cell surface growth factor receptors. Myc is an obligate downstream effector for HER-2/Neu to drive cancer. Female MMTVneu "oncomice" are mated twice to initiate expression from the mouse mammary tumor virus (MMTV) promoter which drives transcription of the Neu/HER2 oncogene in mammary tissue. Mammary tumors arise with a penetrance of >90% in this model system by 5 months of age.

The following examples are provided to illustrate various embodiments of the present invention. They are not intended to limit the invention in any way.

EXAMPLE 1:

Novel IDO inhibitors

A variety of compounds were screened for their

efficacy as IDO inhibitors. Certain compounds were screened in a biochemical assay as follows. IDO cDNAs were expressed in bacteria as his-tagged proteins and purified as previously described (Littlejohn et al. (2000) Prot. Exp. Purif. **19**:22-29). Briefly, the purified IDO was incubated with substrate and varying amounts of the IDO inhibitor candidate. The fluorescence of the reaction mixture was measured to determine the efficacy of the candidate inhibitor because a product of the reaction, kynurenine, is fluorescent. The results of the *in vitro* biochemical screen are depicted in Figure 1.

The candidate compounds were also screened in a cell-based assay (for similar assay see Munn et al. (1999) J. Exp. Med. **189**:1363-1372). Briefly, human 293/Phoenix cells were transiently transfected with human IDO or TDO cDNA expression vectors. The candidate compounds were added to the transfected cells at various concentrations. Kynurenine was quantitated in tissue culture media using a fluorescence-based protocol. The results from these experiments are presented in Figures 2-4.

As noted in these figures, the most potent inhibitors identified are a set of thiohydantoin derivatives of indoleamine. Figure 3 provides results using these particular inhibitors. The most potent of these inhibitors, methyl-TH-DL-trp, displayed an inhibition of IDO activity 2.7 times greater than 1MT at a concentration of 250 μM (Figure 4).

In addition to the thiohydantoin derivatives of indoleamine, a group of natural products was screened. Interestingly, effective inhibitors from this group were compounds from foods with cancer preventitive properties (e.g. cruciferous vegetables). Brassinin, a compound found in Chinese cabbage, was scored as the most potent compound among the natural products determined to be IDO

inhibitors (Figure 2A).

The toxicity of certain screened compounds was also examined. As seen in Figure 5, most IDO inhibitory compounds are not intrinsically growth inhibitory or cytotoxic to neoplastically transformed breast or prostate cancer cells (Fig. 5).

EXAMPLE 2:

Combinatorial treatment of tumors with an IDO inhibitor and cytotoxic chemotherapeutic agent

MMTVneu "oncomice" bearing similarly sized tumors of ~150 mm^3 were randomly assigned to control or experimental treatment groups. Control mice were implanted with placebo time-release pellets (Innovative Research, Inc., Sarasota, FL). Experimental groups of mice were (1) implanted with 1MT-containing time-release pellets, (2) treated with paclitaxel (Taxol®) or other cytotoxic agents, or (3) implanted with 1MT-containing time-release pellets and treated with paclitaxel or other cytotoxic agents. The time-release pellets are comprised of a copolymer which is inert and gradually dissolves and breaks down to a non-toxic substance that remains largely localized during the course of the experiment. Time-release pellets impregnated with 1MT release a dose of 10 mg/day for a period of up to 14 days as documented by the commercial vendor (Innovative Research, Inc., Sarasota, FL). Two pellets per mouse were implanted to deliver a total dose of 20 mg/day. Therefore, for a 25 g mouse the total dose is 800 mg/kg/day or 280 mg over a 14 day period. Steady-state levels were reached within 12-24 hours and are maintained throughout the entire period based on the manufacturer's specifications. The delivered dose is effective at eliciting allogenic

conceptus rejection (A. Muller, J.B. DuHadaway, G.C. Prendergast, unpublished results) as described by Munn et al. (Science 281:1191-1193, 1998).

Time-release pellets were introduced subcutaneously on the backs of mice anesthetized by intramuscular injection of ketamin/rompun. Blunt dissection with a hemostat is used to separate the skin from the underlying muscle to create a subcutaneous pocket. One or two biodegradable slow release pellets were implanted within this pocket, rather than directly under the incision in order to prevent mechanical stress and wound dehiscence. The incision was then closed with wound clips. Based on the ability of female mice that have been implanted with placebo time-release pellets to carry pregnancies to term, distress from the procedure appears to be negligible.

The cytotoxic chemotherapeutic agents were prepared and delivered to the mice as follows. Paclitaxel was dissolved in equal volumes of absolute ethanol and the clinically-used solubilizing agent Cremophor® EL. The solution was sonicated up to 30 minutes and stored as a 20 mg/ml stock solution at 4°C for up to one week. Before use, this solution was diluted further at 1:5 with sterile physiological saline. Paclitaxel formulated in this manner was administered to mice by a single bolus intravenous (i.v.) injection into the tail vein. Mouse tails can be warmed to facilitate identification and injection of the vein. The maximum tolerated dose (MTD) of paclitaxel (13.3 mg/kg) was delivered five (5) times during the 2 week experiment on a thrice-weekly schedule (i.e., Friday - pellet implantation; Monday / Wednesday / Friday, Monday / Wednesday - paclitaxel inject; Friday - euthanize animals and harvest tumors for analysis). The MTD of cisplatin (1 mg/kg) was obtained as a clinical preparation in

saline and delivered as a bolus i.v. injection on the same schedule. Control treated mice received only the Cremophor® EL vehicle formulation without paclitaxel.

Figure 6 and Table 1 summarize the findings of the experiments to test the ability of 1MT to cooperate with two cytotoxic agents to cause regression of established tumors in MMTVneu "oncomouse" model. During the two week course of the experiment, an elevation of ~200% in the tumor volume of mock-treated control mice was observed. Treatment of mice with 20 mg/day 1MT, delivered by subcutaneous time-release pellets, retarded but did not block tumor growth. Similarly, treatment of tumor-bearing mice by intravenous injection of paclitaxel or cisplatin at the maximum-tolerated doses retarded but did not block tumor growth. In contrast, the combination of 1MT plus paclitaxel or cisplatin treatment caused tumor regression in the model. Similar results were observed with a reduction of paclitaxel to ~25% the maximum-tolerated dose (data not shown). Inasmuch as the cytotoxic agents employed in these studies are known to be toxic to the very T cells that the IDO inhibitors would allow to be recruited and activated, these results are unexpected in view of the prior art.

	Untreated	1MT only	Taxol only	1MT + Taxol	Cisplatin only	1MT + Cisplatin
Number of Mice	5	5	5	6	3	3
Number of Tumors	5	7	6	9	5	5
Mean	195.1	80.27	139.4	-30.2	91.35	-27.94
Std. Deviation	97.54	73.12	118.1	30.7	118.5	35.1
Std. Error	43.62	27.64	48.2	10.23	53	15.7
Minimum	122.2	0	20	-78.4	-26.53	-67.86
25% Percentile		25	40.25	-56.5		
Median	134.4	72.87	130.4	-23.44	40	-30.56
75% Percentile		130.4	247.6	-6.445		
Maximum	336.5	215	360.8	12.5	255.6	14.29
Lower 95% CI	73.95	12.65	15.52	-53.8	-55.79	-71.52
Upper 95% CI	316.2	147.9	263.3	-6.605	238.5	15.64

Table 1

Statistical analysis of the tumors of MMTVneu mice after various treatments. Numbers are provided as percent change in tumor volume as compared to the tumor volume prior to treatment. Lower and upper 95% Cl indicate lower and upper 95% confidence limits.

Histological and immunohistochemical analysis of tumor sections isolated from the control and experimental cohorts revealed dramatic changes only in the tumor tissues from the mice treated with the combinatorial regiment. Most notably, evidence of pronounced hemorrhage, apoptosis, and infiltration of CD3-positive T cells was seen in the mice that received the combinatorial regiment (data not shown). In conclusion, the combined application of 1MT with cytotoxic agents was efficacious in eliciting regression of established breast tumors in the MMTVneu "oncomouse" model system.

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

What is claimed is:

1. A method for inhibiting tumor growth in a patient comprising administration of an effective amount of a pharmaceutical composition comprising at least one inhibitor of indoleamine 2,3-dioxygenase selected from the group consisting of indole 3-carbinol, 3,3'-diindolylmethane, brassinin, epigallocatechin gallate, 5-Br-4-Cl-indoxyl 1,3-diacetate, 9-vinylcarbazole, acemetacin, 5-bromo-DL-tryptophan, 5-bromoindoxyl diacetate, phenyl-TH-DL-trp, propenyl-TH-DL-trp, and methyl-TH-DL-trp.

2. A method of treating cancer in a patient comprising administration of an effective amount of a pharmaceutical composition comprising at least one inhibitor of indoleamine 2,3-dioxygenase and at least one cytotoxic chemotherapeutic agent.

3. The method of claim 2, wherein said at least one inhibitor of indoleamine 2,3-dioxygenase is selected from the group consisting of 1-methyl-DL-tryptophan, β-(3-benzofuranyl)-DL-alanine, β-(3-benzo(b)thienyl)-DL-alanine, 6-nitro-L-tryptophan, indole 3-carbinol, 3,3'-diindolylmethane, brassinin, epigallocatechin gallate, 5-Br-4-Cl-indoxyl 1,3-diacetate, 9-vinylcarbazole, acemetacin, 5-bromo-DL-tryptophan, 5-bromoindoxyl diacetate, phenyl-TH-DL-trp, propenyl-TH-DL-trp, and methyl-TH-DL-trp.

4. The method of claim 2, wherein said at least one cytotoxic chemotherapeutic agent is selected from the group consisting of placitaxel (Taxol®), cisplatin, docetaxol, carboplatin, vincristine, vinblastine, methotrexate, cyclophosphamide, CPT-11, 5-fluorouracil

(5-FU), gemcitabine, estramustine, carmustine, adriamycin (doxorubicin), etoposide, arsenic trioxide, irinotecan, and epothilone derivatives.

5. A pharmaceutical composition for the treatment of cancer suitable for administering to a patient in need thereof comprising an effective amount of at least one indoleamine 2,3-dioxygenase inhibitor selected from the group consisting of indole 3-carbinol, 3,3'-diindolylmethane, brassinin, epigallocatechin gallate, 5-Br-4-Cl-indoxyl 1,3-diacetate, 9-vinylcarbazole, acemetacin, 5-bromo-DL-tryptophan, 5-bromoindoxyl diacetate, phenyl-TH-DL-trp, propenyl-TH-DL-trp, and methyl-TH-DL-trp in a pharmaceutically acceptable carrier medium.

6. A pharmaceutical composition for the treatment of cancer suitable for administering to a patient in need thereof comprising an effective amount of at least one indoleamine 2,3-dioxygenase inhibitor and an effective amount of at least one cytotoxic chemotherapeutic agent in a pharmaceutically acceptable carrier medium.

7. The pharmaceutical composition of claim 6, wherein said at least one indoleamine 2,3-dioxygenase inhibitor is selected from the group consisting of 1-methyl-DL-tryptophan, β-(3-benzofuranyl)-DL-alanine, β-(3-benzo(b)thienyl)-DL-alanine, 6-nitro-L-tryptophan, indole 3-carbinol, 3,3'-diindolylmethane, brassinin, epigallocatechin gallate, 5-Br-4-Cl-indoxyl 1,3-diacetate, 9-vinylcarbazole, acemetacin, 5-bromo-DL-tryptophan, 5-bromoindoxyl diacetate, phenyl-TH-DL-trp, propenyl-TH-DL-trp, and methyl-TH-DL-trp.

8. The pharmaceutical composition of claim 7,

wherein said at least one indoleamine 2,3-dioxygenase inhibitor is 1-methyl-DL-tryptophan.

9. The pharmaceutical composition of claim 6, wherein said at least one cytotoxic chemotherapeutic agent is selected from the group consisting of placitaxel (Taxol®), cisplatin, docetaxol, carboplatin, vincristine, vinblastine, methotrexate, cyclophosphamide, CPT-11, 5-fluorouracil (5-FU), gemcitabine, estramustine, carmustine, adriamycin (doxorubicin), etoposide, arsenic trioxide, irinotecan, and epothilone derivatives.

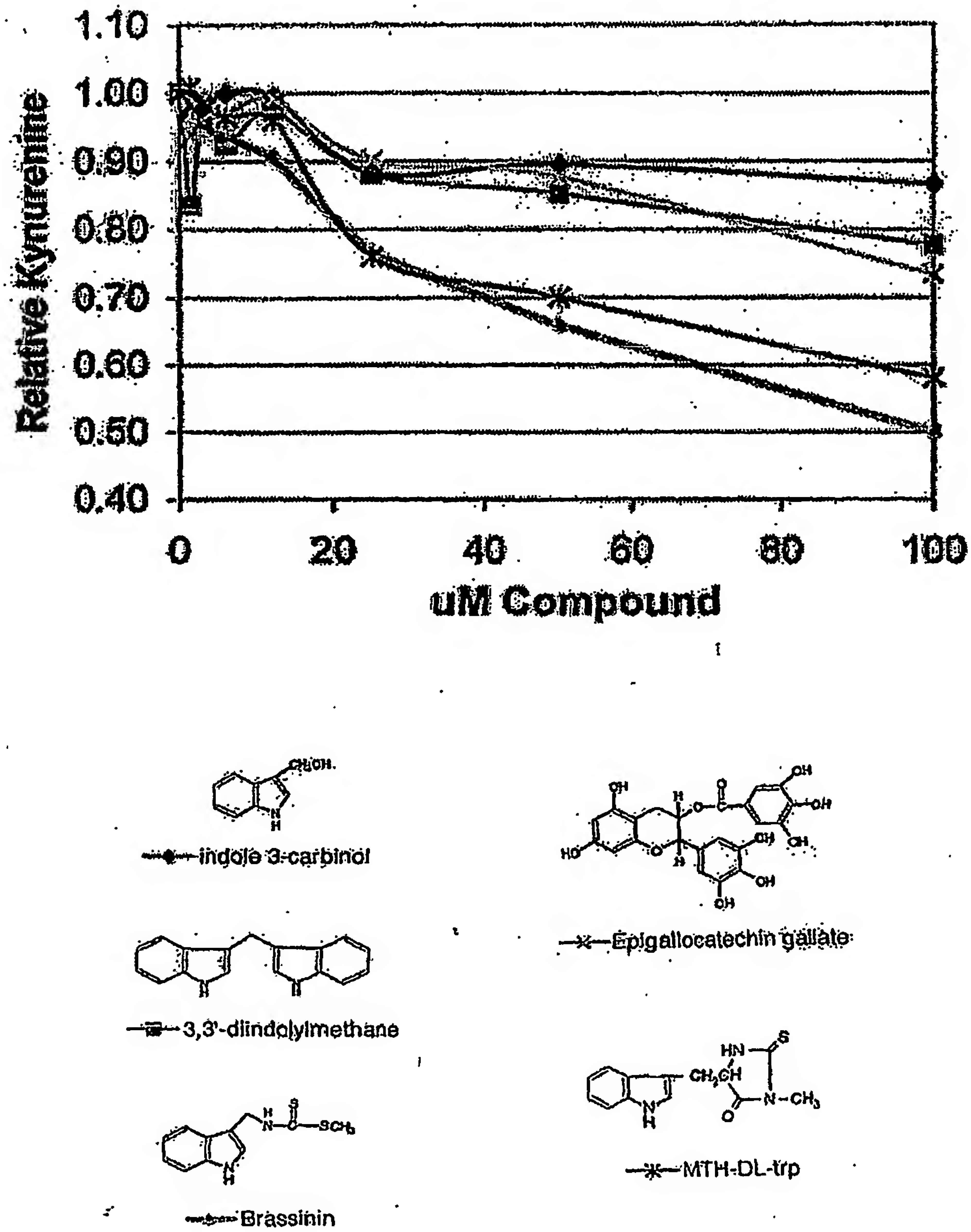
10. The pharmaceutical composition of claim 9, wherein said at least one cytotoxic chemotherapeutic agent is paclitaxel.

11. The pharmaceutical composition of claim 6, wherein said at least one cytotoxic chemotherapeutic agent is paclitaxel and said at least one indoleamine 2,3-dioxygenase inhibitor is 1-methyl-DL-tryptophan.

ABSTRACT

Compositions and methods are provided for a combinatorial drug strategy for the treatment of malignancy.

Figure 1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Relative Kynurenine
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20
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3,3'-diindolylmethane
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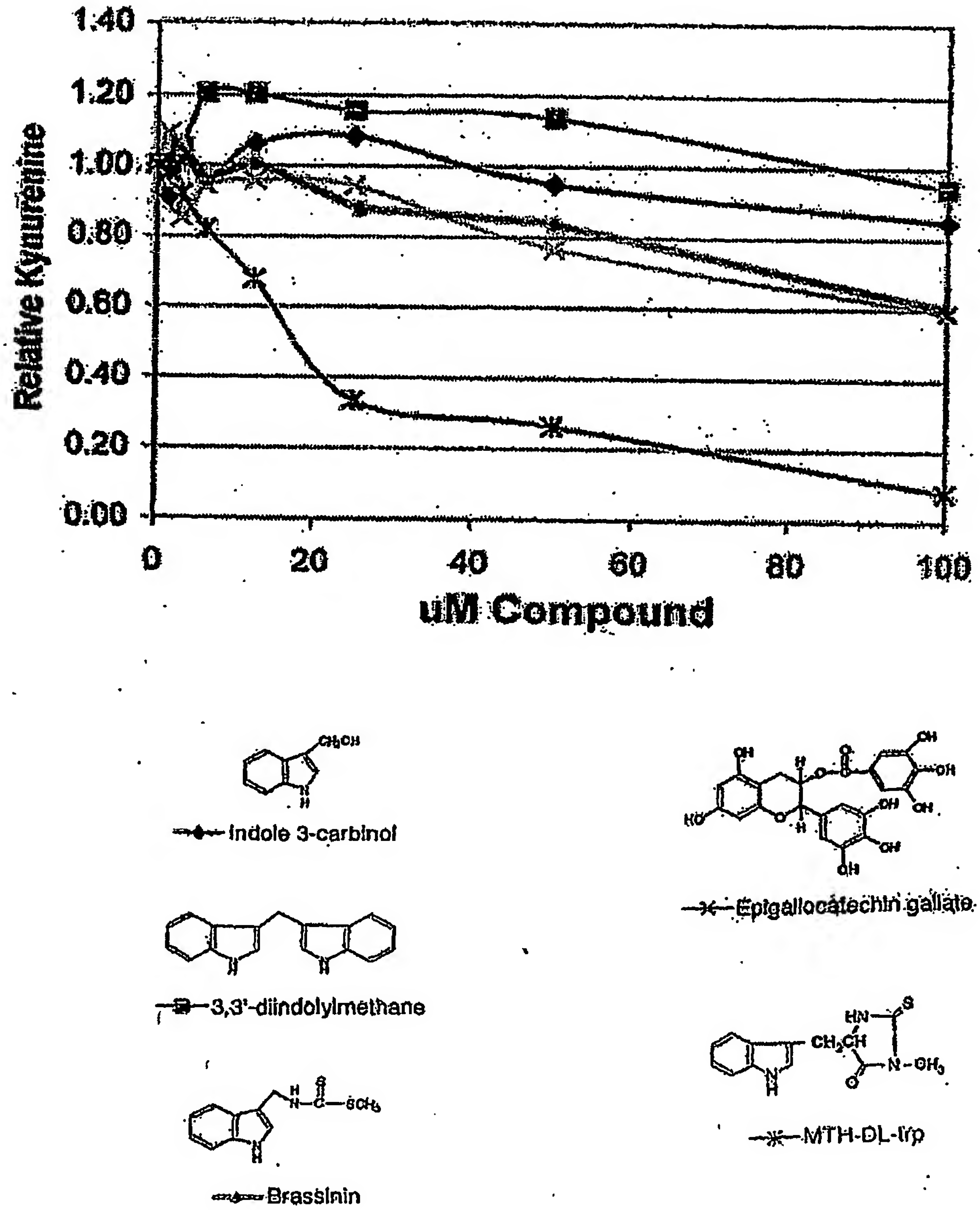
Epigallocatechin gallate
MTH-DL-trp

Fig. 2A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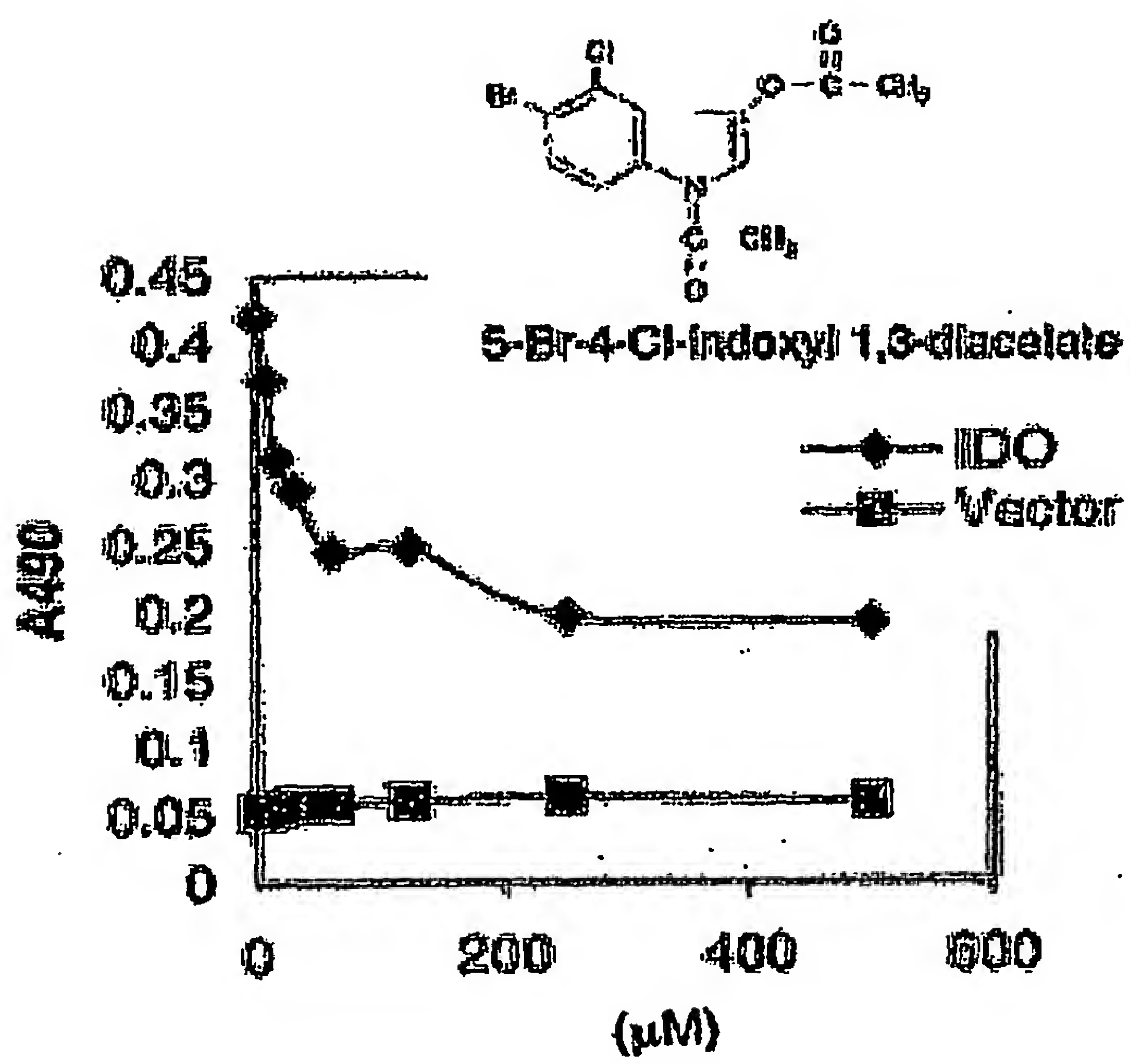
3,3'-diindolylmethane
Brassinin
Epigallocatechin gallate
MTH-DL-trp

Fig. 2B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IDO
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0.35
0.3
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0.15
0.1
0.05
0
0
200
400
600
(μM)

Figure 3

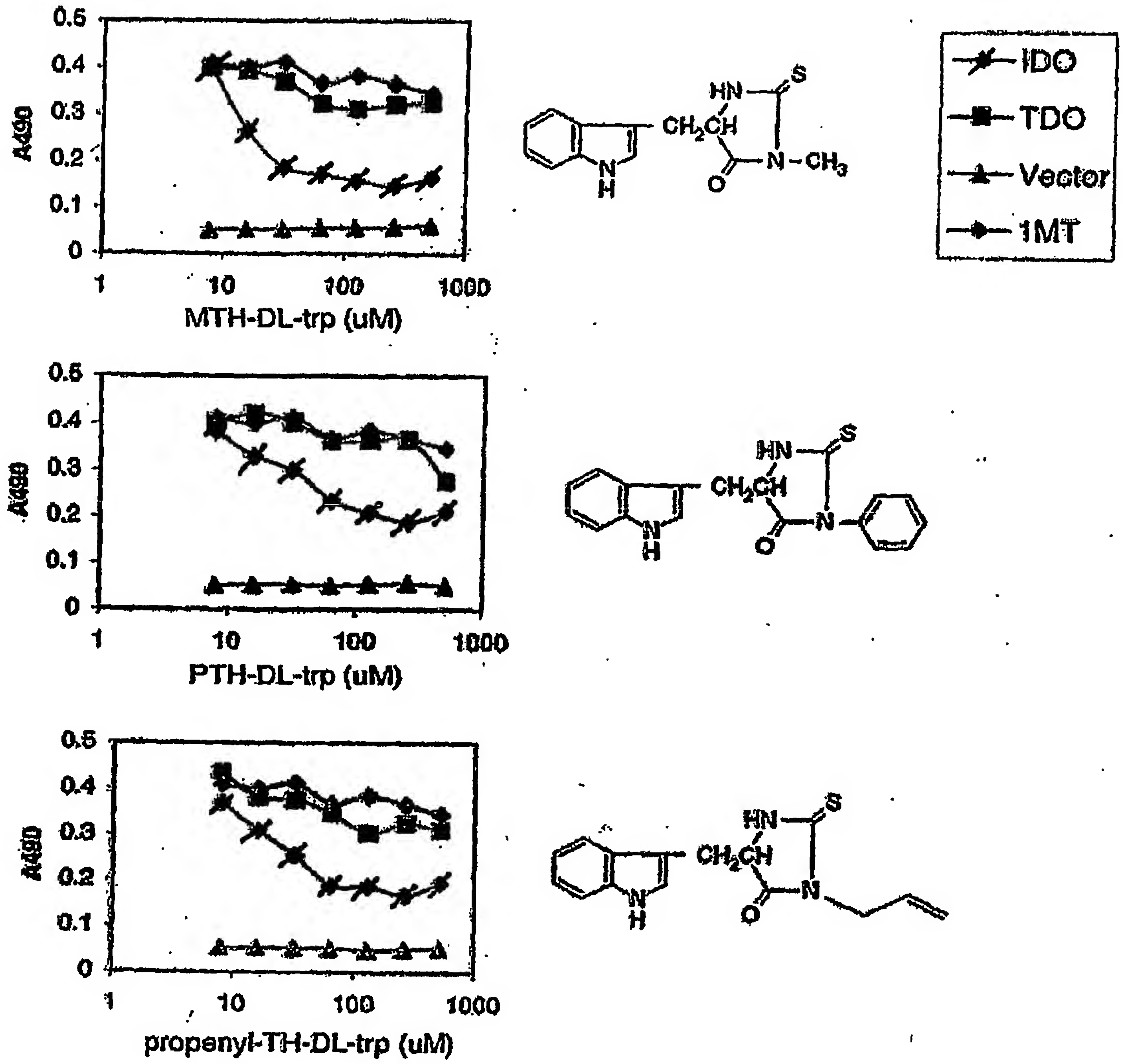
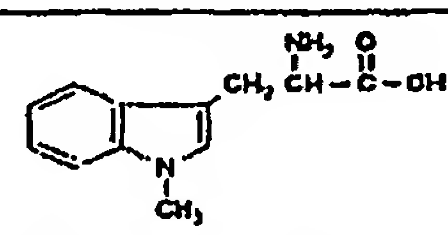
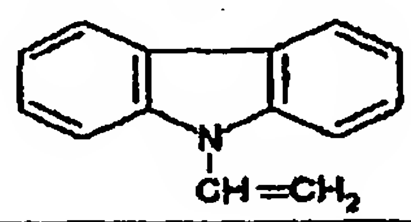
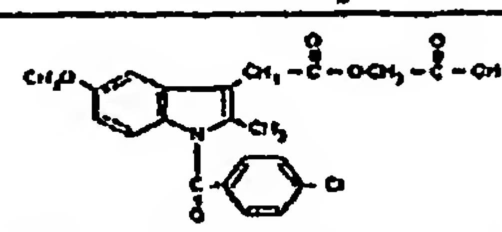
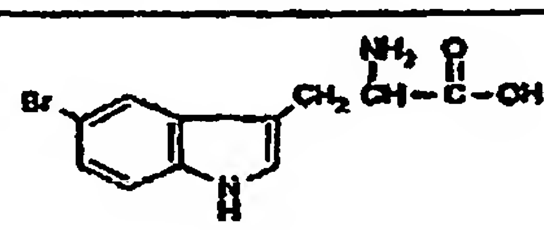
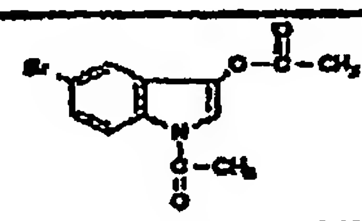

IDO
TDO
Vector
1MT
A490
0.5
0.4
0.3
0.2
0.1
0
1
10
100
1000
MTH-DL-trp (uM)
PTH-DL-trp (uM)
propenyl-TH-DL-trp (uM)
HN
S
CH$_2$CH
N-CH$_3$
O
N
H

Figure 4

Compound name	Structure	IDO inhibition @ 250 μM	TDO inhibition @ 250 μM
1-DL-Methyl-Tryptophan		25.32%	4.74%
9-Vinylcarbazole		22.94%	19.33%
Acemetacin		30.25%	N.D.
5-Bromo-DL-tryptophan		31.49%	18.05%
5-Bromoindoxyl diacetate		59.72%	N.D.

Thiohydantoin (TH) derivatives of indoleamine

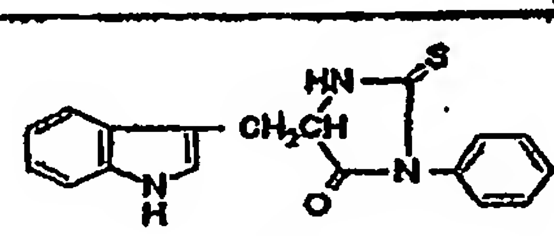
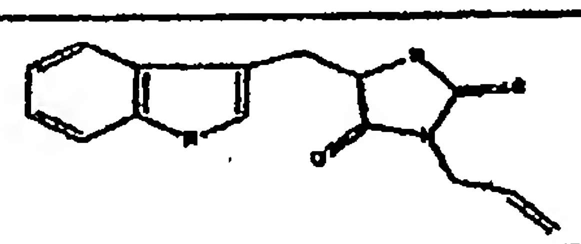
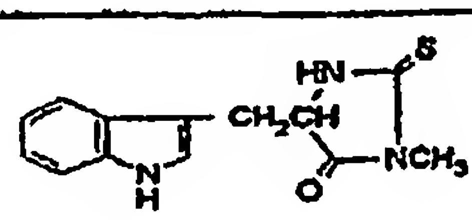
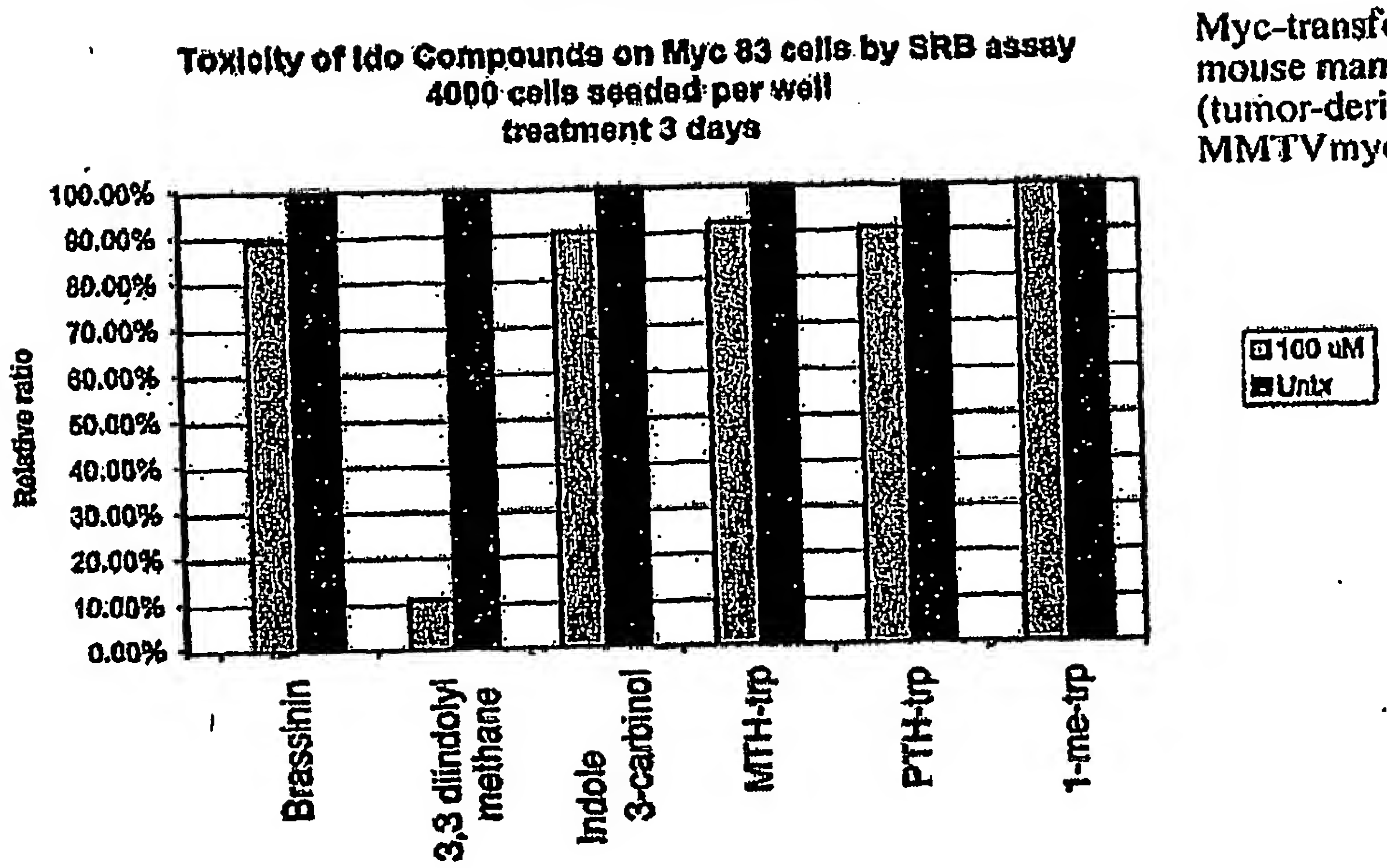
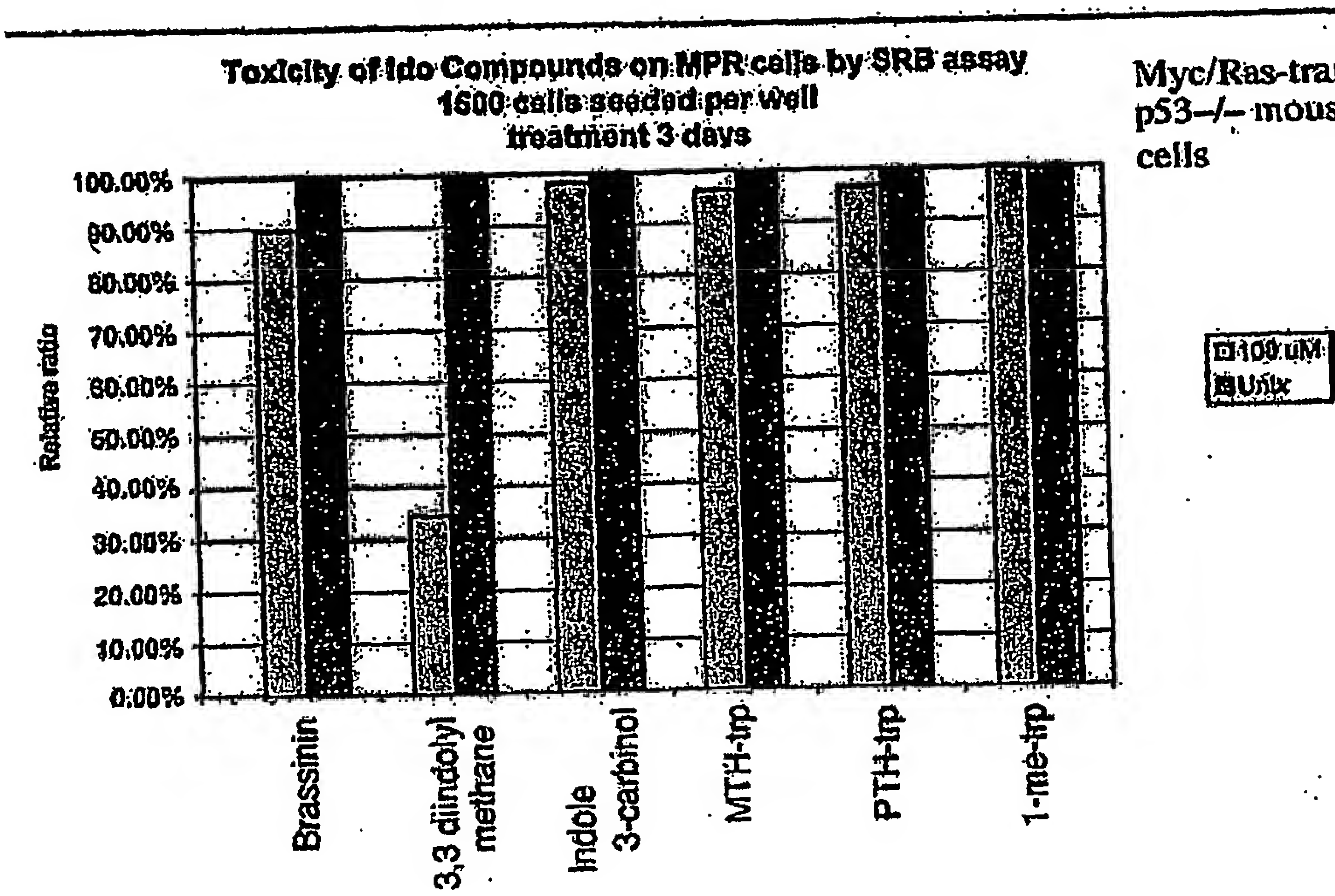
phenyl-TH-DL-trp (PTH-trp)		56.95%	17.83%
propenyl-TH-DL-trp (propTH-trp)		62.72%	25.17%
methyl-TH-DL-trp (MTH-trp)		68.40%	27.05%

Figure 5

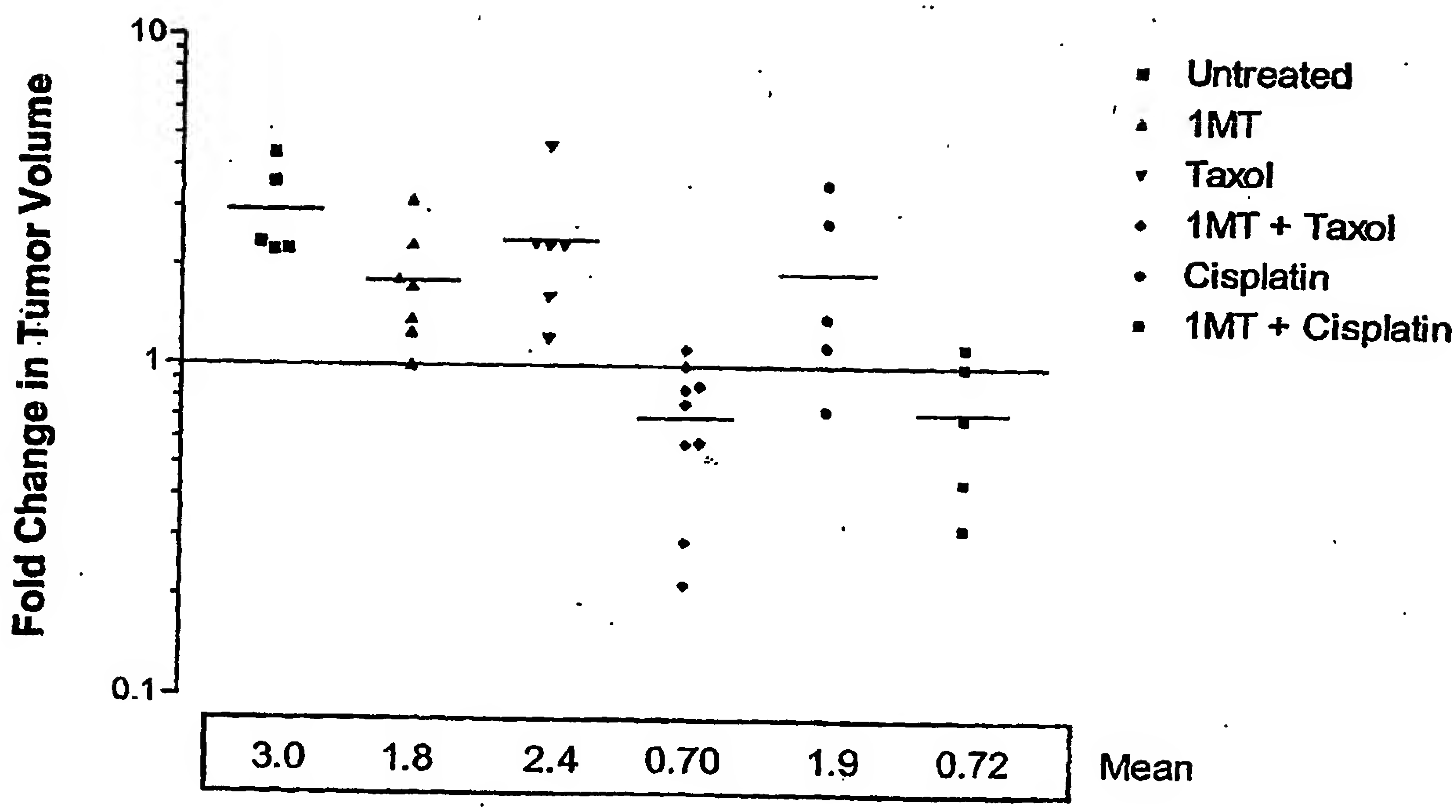


Myc-transformed mouse mammary cells (tumor-derived from MMTVmyc mouse)



Myc/Ras-transformed p53−/− mouse prostate cells

Figure 6


10
1
0.1
Fold Change in Tumor Volume
Untreated
1MT
Taxol
1MT + Taxol
Cisplatin
1MT + Cisplatin
3.0
1.8
2.4
0.70
1.9
0.72
Mean